Selenate and selenite uptake, accumulation and toxicity in *Lemna minuta*

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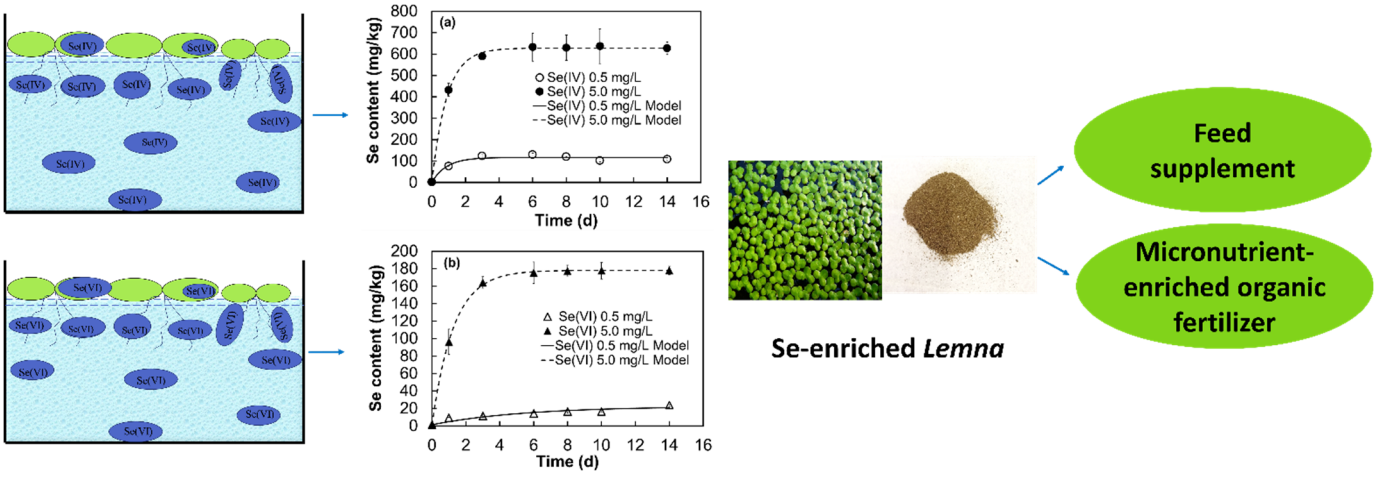
Graphical abstract



Abstract

The kinetics of Se uptake and toxicity to *Lemna* were studied over a period of 14 days of exposure to Se(IV) or Se(VI). The growth of *Lemna* stopped immediately after exposure to 5.0 mg/L of Se(IV) or Se(VI). The content of chlorophyll and phaeopigments of *Lemna* exposed to 5.0 mg/L of Se(IV) was 2-3 times less than in the control after 3 d exposure. *Lemna* took up Se rapidly within the first 3 d. The Se content in *Lemna* along with the exposure time fitted well the two-compartment and the hyperbolic model, which demonstrates that the mechanism of Se(IV) and Se(VI) uptake in *Lemna* is not only through passive diffusion, but also through other processes such as ion channel proteins or transporters. The kinetic BCFs was 231 and 42 for 0.5 mg/L Se(IV) and Se(VI) exposure, respectively. The uptake rate of *Lemna* reached 263 mg/kg/d and 28 mg/kg/d in the Se(IV) and Se(VI) treatments, respectively. This study showed that Se(IV) has a faster accumulation rate than Se(VI), but a higher toxicity, indicating *Lemna* could be a good candidate to remove Se(IV) from water, producing Se-enriched biomass which may eventually also be considered for use as Se-enriched feed supplement or fertilizer.

Keywords: Duckweed, selenium, Se uptake, toxicokinetic model

Introduction

Selenium (Se) is an essential micronutrient for humans and animals (Hatfield et al., 2014). However, the thresholds of Se in the environment that differentiate among deficiency, suitability and toxicity are very narrow (Fordyce, 2013). Increasing anthropogenic activities such as mining, agriculture and industrial manufacturing produce wastewaters containing Se, for example those of coal (0.4-1500 µg/L) and gold (1700-33000 µg/L) mining, flue gas desulfurization process water (1.0-10000 µg/L) and agricultural drainage (140-1400 µg/L) (Lemly, 2004; Tan et al., 2016). These can result in elevated Se concentrations in the receiving water bodies that exceed the current chronic aquatic life criteria in lentic (1.5 µg/L) and lotic (3.1 µg/L) waters adopted by the United States Environmental Protection Agency (USEPA, 2016). For instance, Se concentrations of 7-14 µg/L were found in Hyco Lake (North Carolina, USA) near the effluent source of a power plant and 9.6 µg/L Se was measured in Elk River (British Columbia, Canada) near coal mines (Young et al., 2010). Additionally, Flanders (Belgium) has adopted a surface water quality standard of a maximum of 3.0 µg/L of total Se (Valarem II, 1995), while the Canadian Council of Ministers of the Environment has established a limit of 20-50 µg/L of Se for irrigation water (Etteieb et al., 2020).

Phytoextraction is an environmentally friendly method to remove excess contaminants and nutrients from water. Duckweed (*Lemna* sp.), as an aquatic floating plant, is a good candidate for the treatment of wastewaters because of its fast growth rates, easy harvest, and simple structure (Panfili et al., 2017). Additionally, duckweed containing high levels of protein (around 20-40%) and starch can also be explored as a valuable protein and carbohydrate source for animal feed (Zhong and Cheng, 2016). Particularly, Se taken up by plants is easily converted into organic Se compounds, such as the selenoamino acids Se-cystine ($SeCys_2$) and selenomethionine (SeMet), which have a benefit for human and animal nutrient intake (Terry et al., 2000). Additionally, the use of the aquatic plant duckweed for the removal of Se from

contaminated water and the production of Se-enriched biomass for use as an animal feed supplement or micronutrient fertilizer would contribute to the drive for the circular economy. However, there is limited information about Se accumulation in duckweed and the physiological and biochemical response of duckweed to Se oxyanions.

Selenium compounds exist in nature in five redox states: Se(-II), Se(0), Se(II), Se(IV) and Se(VI) (Terry et al., 2000). Different chemical forms of Se have different properties regarding bioavailability, mobility, and toxicity. Selenium enters freshwater mainly as selenite (Se(IV)) and selenate (Se(VI)) oxyanions due to their high solubility. These are the two major forms of toxic Se in the ecosystem, because the two Se species are readily taken up by aquatic plants or animals and metabolized to organic Se compounds, resulting in Se bioaccumulation and toxicity to organisms (Mechora et al., 2015). High Se concentrations in plants can cause symptoms of toxicity such as growth inhibition, leaf chlorosis and premature death. For instance, Zhong et al. (2016) reported negative impacts of Se(IV) on the chlorophyll fluorescence and starch content of the duckweed *Landoltia punctata* after exposure to Se(IV) concentration higher than 3.2 mg/L. Carvalho and Martin (2001) recorded that the fresh weight of the duckweed *Lemna obscura* Aust. decreased from 50 to 20 mg when the Se(IV) dose was increased from 1.0 to 20 mg/L in the medium. Ohlbaum et al. (2018) found that the chlorophyll *a* content decreased from 0.36 to 0.13 mg/g fresh weight for *Lemna* when increasing the Se(VI) concentration from 0.05 to 0.5 mg/L in the leachate of a seleniferous soil. Mechora et al. (2015) studied the response of duckweed to various Se(IV) concentrations and found that *Lemna minor* L. was dying at the highest Se(IV) concentration (10 mg/L). However, which of the inorganic Se forms, Se(IV) or Se(VI), has a higher aquatic toxicity for specific organisms is not clear: some studies indicated that Se(IV) is more toxic (Li et al., 2020), while the opposite was found in other studies (Kroflic et al., 2016). The difference may depend on the exposure time, plant species or ambient

conditions. Therefore, it is necessary to characterize the toxicity of Se to duckweed before implementing it in phytoextraction.

Metal accumulation affects the cycling of pollutants and micronutrients in aquatic systems and impacts the ecosystem's health. Because of the complexity of metal uptake, detoxification and excretion processes in plants, it is difficult to directly predict all mechanisms and routes of bioaccumulation. This has led to the development of theoretical kinetic models to predict metal uptake and retention in both terrestrial and aquatic species (Clason et al., 2003). First-order kinetics are characteristic of a compartment model where the uptake rate of a chemical is linear with exposure concentration. When passive transport processes like diffusion are the main uptake mechanism, first-order kinetic models make a reasonable prediction over a wide range of exposure concentrations. However, if metal uptake is facilitated not only by diffusion, but also by ion channel proteins or carriers, the relationship between metal uptake and exposure concentrations will follow a saturation curve, indicating that first-order kinetic models are not suitable at the higher exposure concentrations. In this situation, the non-linear two-compartment and the hyperbolic model might be applied to metal bioaccumulation over a wide exposure range. For instance, Templeman and Kingsford (2015) evaluated the accumulation of Cu and Zn in the jellyfish *Cassiopea maremetens* through a two-compartment model. Clason *et al*. (2003) showed that the two-compartment and hyperbolic toxicokinetic models could simulate the bioaccumulation of the heavy metals Cd, Pb, Cu and Zn in the Antarctic amphipod *Paramoera walker*. Selenium, as a metalloid, to some extent has the same characteristics as metals, but there has been no research evaluating the theoretical kinetic models of Se accumulation in plants and quantifying Se uptake rates of duckweed.

This study aimed to (1) assess the dynamic effect of Se on the physiology of duckweed during a 14 d incubation period under Se(IV) or Se(VI) exposure, (2) quantify the capacity of duckweed to accumulate the two forms of Se from aqueous medium, and (3) evaluate if the

toxicokinetic models can predict bioconcentration patterns of aqueous Se(IV) and Se(VI) in duckweed.

Materials and methods

Lemna source and cultivation

Duckweed (*Lemna minuta*) was randomly collected from a natural freshwater canal in Delft (The Netherlands), and cultivated in modified Hoagland solution (Ohlbaum et al., 2018) at pH 6 to acclimatize for seven days in a greenhouse. The modified Hoagland solution contained: 118 mg/L $Ca(NO_3)_2{\cdot}4H_2O$, 5.0 mg/L KNO_3, 5.0 mg/L $MgSO_4{\cdot}7H_2O$, 0.3 mg/L $FeSO_4{\cdot}7H_2O$, 0.15 mg/L $MnSO_4{\cdot}7H_2O$, 8.0 µg/L $CuSO_4{\cdot}5H_2O$, 300 µg/L H_3BO_3, 1.28 µg/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.79 µg/L $NaWO_4{\cdot}2H_2O$, 22 µg/L $ZnSO_4$, 5.0 µg/L $NiSO_4{\cdot}7H_2O$ and 4.0 µg/L $CoCl_2{\cdot}6H_2O$. The temperature in the greenhouse varied between 25 and 30 °C and light was provided with a minimum intensity of 100 µmol photons/m^2/s.

Kinetics of Se accumulation and tolerance

After seven days of incubation, 1.0 g (wet weight) of *Lemna* was transplanted to 100 mL of Hoagland solution supplemented with 0.5 or 5.0 mg/L of Se(IV) or Se(VI) added as sodium selenite (Na_2SeO_3) or sodium selenate (Na_2SeO_4), respectively. Medium without Se served as control. Three replicate pots were prepared for each treatment and incubation period (0, 1, 3, 6, 8, 10, and 14 d). The total production of *Lemna* in each pot was harvested at every time point for analysis. The harvested biomass was washed with deionized water (DI) and analyzed for growth indicators (fresh weight), tolerance index of roots (root length), total Se content, and photosynthetic pigments concentrations.

Analytical methods

For total Se determination, the plants were oven-dried at 60 °C until constant weight and then digested with 10 mL concentrated HNO_3 in a microwave (CEM Mars 5, Matthews, NC, USA). The digested solution was diluted with DI water and analyzed for the total Se content using an atomic absorption spectrophotometer coupled to a graphite furnace (GF-AAS, Thermo Elemental Solaar MQZ, GF95, Thermo Fisher Scientific, Waltham, MA, USA). The multi-element standard solution as quality control was always analyzed along with each batch samples to evaluate the accuracy of the total Se determination.

Chlorophyll α and β and their decomposition products phaeopigments α and β were determined following the procedure of Wintermans and De Mots (1965). 0.1 g fresh weight of *Lemna* was ground in a mortar with 5 mL ethanol (96%), transferred to a centrifuge tube and left overnight in dark conditions for extraction. The samples were centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 750, 665 and 649 nm in a UV-Vis spectrophotometer (Lambda 365 UV/Vis, PerkinElmer, Waltham, MA, USA) for the measurement of chlorophyll α and β. Afterwards, 0.5 mL of 0.06 M HCl was added to 3 mL of supernatant for acidification. The absorbance of the acidified supernatant was determined at 750, 666 and 655 nm for the measurement of phaeopigments α and β.

Data analysis

Statistical differences of the data were analyzed with the ANOVA and Duncan's multiple comparison tests in SPSS 20.0.

The tolerance index of the *Lemna* roots was evaluated by measuring the average length of 10 roots of each sample and calculated by the following equation (1):

$$Tolerance\ index = \frac{Average\ root\ length\ of\ sample}{Average\ root\ length\ of\ control} \quad (1)$$

The content of chlorophyll α and β, and phaeopigments α and β were calculated according to the following equations (2), (3), (4) and (5):

$$\text{Chlorophyll } \alpha = \frac{[13.70 \times (A665 - A750) - 5.76 \times (A649 - A750)] \times V \times D}{FW \times 1000} \quad (2)$$

$$\text{Chlorophyll } \beta = \frac{[25.80 \times (A649 - A750) - 7.60 \times (A665 - A750)] \times V \times D}{FW \times 1000} \quad (3)$$

$$\text{Phaeopigments } \alpha = \frac{[24.50 \times (A666a - A750a) - 9.32 \times (A655a - A750a)] \times V \times D}{FW \times 1000} \quad (4)$$

$$\text{Phaeopigments } \beta = \frac{[36.97 \times (A655a - A750a) - 18.48 \times (A666a - A750a)] \times V \times D}{FW \times 1000} \quad (5)$$

where: V = extraction volume (mL), D = sample dilution factor, FW = fresh weight of sample (g), and A655a, A666a, A750a = absorbance at 655, 666 and 750 nm after acidification, respectively.

The time course of the Se uptake was evaluated by the two-compartment model (Clason et al., 2004a; 2004b), in which the Se exposure and the *Lemna* plants were considered as the first and second compartments, respectively. The model parameters for uptake and clearance were estimated by equation (6), taking into account only data from the uptake phase and using non-linear iterative least square methods:

$$C_A = C_0 + C_w \frac{K_a}{K_b}(1 - e^{-K_b t}) \quad (6)$$

where: C_A is the Se concentration in *Lemna* (mg/kg), C_0 is the background Se concentration in *Lemna* from the control (mg/kg), C_w is the Se exposure concentration (mg/L), K_a is the rate constant of Se uptake (mg/kg/d) and K_b (mg/kg/d) is the rate constant for clearance, which occurs in parallel with the uptake.

For the two-compartment model, the bioconcentration factor (*BCF*) at the theoretical equilibrium was calculated with the following equation (7):

$$BCF = \frac{K_a}{K_b} \quad (7)$$

where: K_a and K_b are derived from the two-compartment model (equation (6)).

Alternatively, the time course of Se uptake was analyzed with a hyperbolic model (Clason et al., 2003) estimated with the following equation (8):

$$C_A = C_0 + \frac{C_{max}t}{t_{max/2}+t} \quad (8)$$

where: C_A is the Se concentration in the *Lemna* (mg/kg), C_0 is the background Se concentration in *Lemna* from the control (mg/kg), C_{max} is the maximum Se concentration in plants at theoretical equilibrium (mg/kg) and $t_{max/2}$ is the time to reach half of the C_{max} (d).

Results and discussion

Effect of Se(IV) and Se(VI) on the growth rate of *Lemna*

The biomass production of *Lemna* significantly decreased upon Se application compared to the control (Figure 1). The fresh biomass of *Lemna* significantly increased with incubation time, but at a slower growth rate under Se exposure when compared with the control. After 14 d of incubation, the biomass of *Lemna* increased up to 1.7 and 1.5 g in the control and the 0.5 mg/L Se treatments (both Se(IV) and Se(VI)), respectively. The fresh biomass in the 5.0 mg/L Se amendments remained unchanged during the whole incubation period. This indicated that Se toxicity (both Se(IV) and Se(VI)) stunts *Lemna* biomass growth, especially at 5.0 mg/L Se application. Similarly, Li et al. (2020) found that the dry weight of *Azolla cristata* decreased significantly (from 100 mg to 80 mg) ($P < 0.01$) when the Se(IV) exposure increased to 0.5 mg/L in the medium. The biomass of stem and leaves formed during Se exposure were markedly reduced in alfalfa (*Medicago sativa* cv.) exposed to 100 and 900 μM Se (~7.9 mg/L and 71 mg/L) compared to the non-exposed controls (Dai and Jia, 2017). Ohlbaum et al. (2018) found

that 0.5 mg/L of Se(VI) in Hoagland solution caused the death of approximately 5% of the exposed *Lemna minor* and *Egeria densa*.

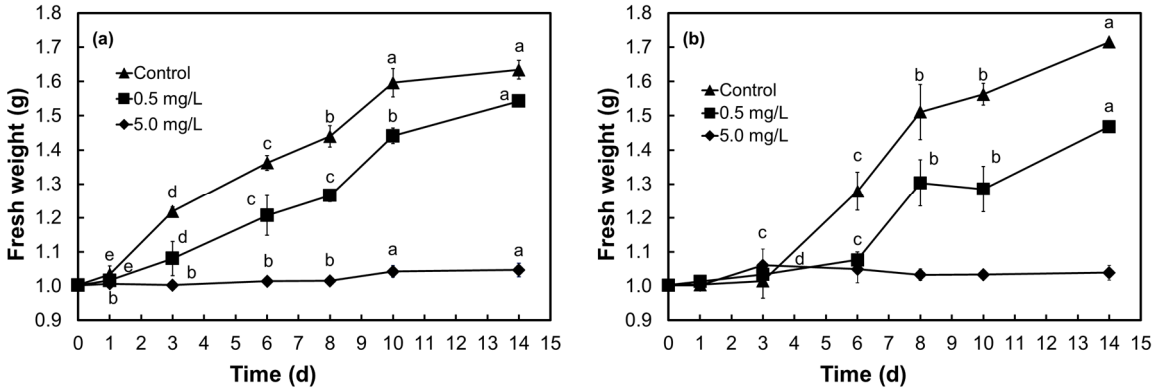


Figure 1. Fresh weight of *Lemna* grown for 14 days on medium containing (a) selenite and (b) selenate. Values are mean ± standard deviation (n=3). Different letters indicate statistically significant differences among treatments with the same Se application according to Duncan's multiple comparison tests ($P < 0.05$).

Effect of Se(IV) and Se(VI) on the *Lemna* root length

Both low and high Se(IV) concentrations caused a significant inhibition in the root growth of *Lemna*, while the low Se(VI) exposure concentration did not show any notable effect, compared with the control (Figure 2). The root length of *Lemna* significantly increased along with the incubation time in the control and low Se exposure. During the 14 d cultivation period, the root length of *Lemna* exposed to 0.5 mg/L Se(IV) and Se(VI) increased in 1.2 cm and 2.1 cm, respectively, which indicated a slower root growth in the Se(IV) medium than in the Se(VI) medium. The growth of the *Lemna* roots stopped immediately when exposed to 5.0 mg/L of Se(IV), and after 3 d of incubation in the 5.0 mg/L Se(VI) medium. The slower growth rate of the roots under Se(IV) exposure confirms the higher Se(IV) toxicity to *Lemna*.

The root tolerance index is the ratio of the root length of a treatment to that of the control (equation (1)) and reflects the tolerance of plants to contaminants. The roots of plants are directly in contact with the contaminant, so the root tolerance index is a sensitive indicator. *Lemna* tolerated higher concentrations of Se(VI) than those of Se(IV), which was also

evidenced by the higher tolerance index in the Se(VI) treatments (Figure 3). Generally, the root tolerance index declined with increasing exposure time to both Se(IV) and Se(VI) medium, which indicated that the root growth under Se exposure becomes slower along with the incubation time.

The higher Se(IV) toxicity can be explained by the different transformation pathways of Se in plants. Se(IV) taken up by plants is easily converted into organic Se (e.g., SeMet and SeCys) and then misincorporated into proteins by replacing cysteine and methionine, resulting in the malformation of proteins and inactivation of enzymes (Sabbagh and Van Hoewyk, 2012). Specifically, the amino acid cysteine is often found at the active site of enzymes and thus, is involved in catalytic reactions. Besides, cysteine is essential for the formation of disulfide bridges, which has an important role to maintain protein function and structure. Given the cysteine's role in proteins, replacing cysteine with SeCys by misincorporating it into plants proteins could impair or misfold proteins, resulting in Se toxicity in plants (Sabbagh and Van Hoewyk, 2012; Van Hoewyk, 2013). This is thought to be the cause of Se toxicity in *Lemna*. While the Se(VI) transformation in plants is a slow and energy-consuming process (Van Hoewyk, 2013). Therefore, Se(VI) taken up by *Lemna* most likely exists as inorganic Se(VI) form (Li et al., 2020). Additionally, oxidative stress is another mechanism of Se toxicity (Van Hoewyk, 2013). Several studies have shown the ability of some cell types to catalyze the bioconversion of SeMet to alternative forms capable of producing superoxides (Ponce et al. 2018). Thus, organic Se forms, such as SeMet, possibly present in *Lemna* exposed to Se(IV) in this study may be metabolized to superoxides, resulting in a higher toxicity of Se(IV) than Se(VI).

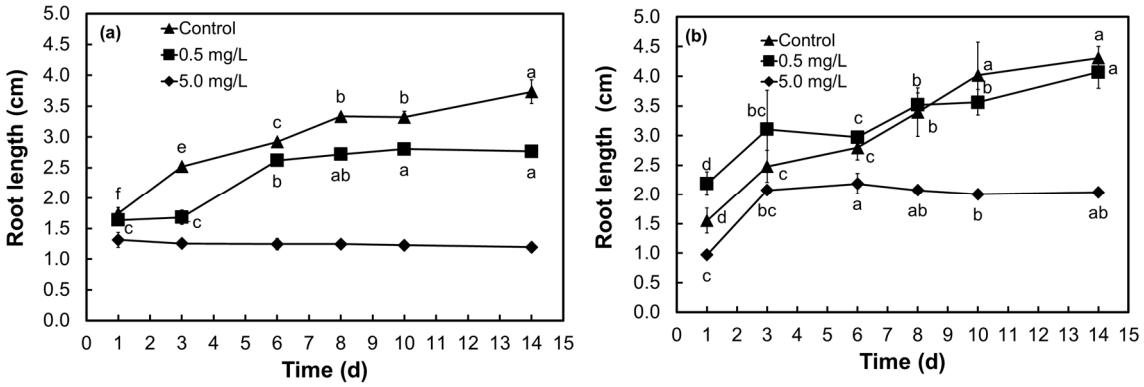


Figure 2. Root length of *Lemna* exposed to medium containing (a) selenite and (b) selenate. Values are mean ± standard deviation (n=3). Different letters indicate statistically significant differences among treatments with the same Se application according to Duncan's multiple comparison tests ($P < 0.05$).

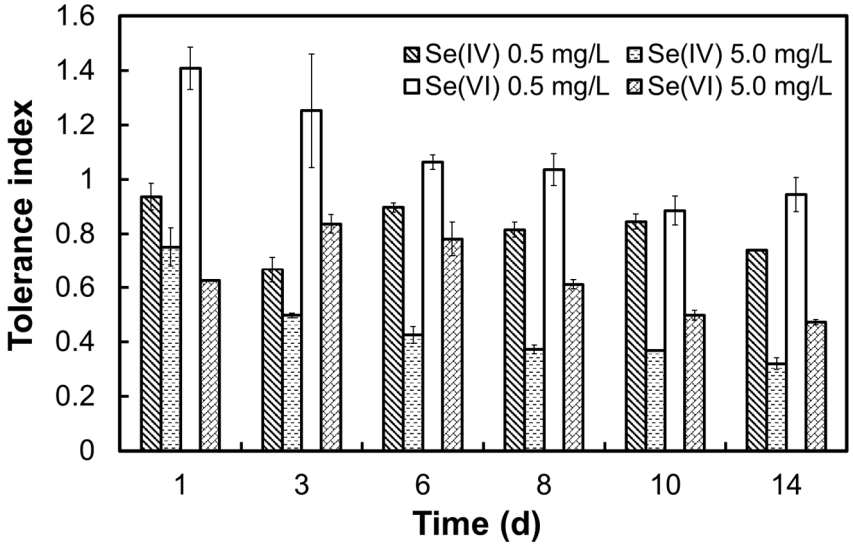


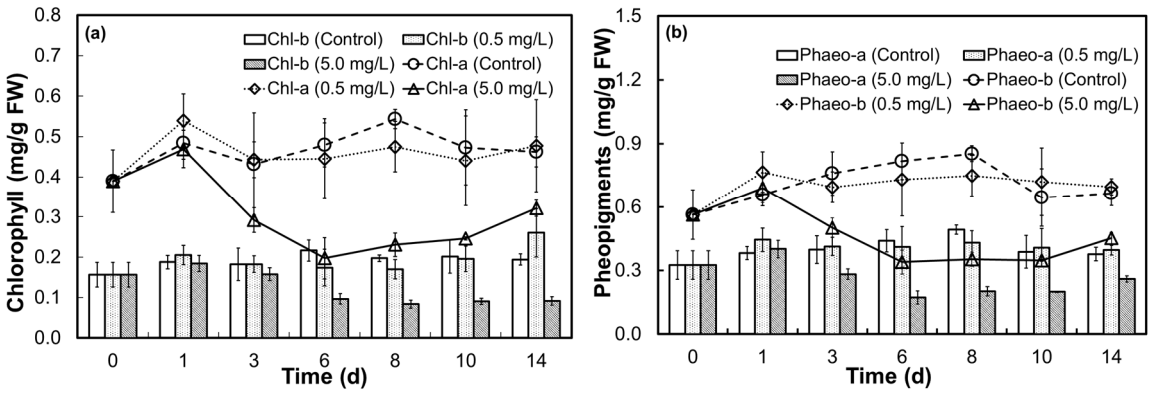
Figure 3. Root tolerance index of *Lemna* after exposure to Se containing medium during 14 days incubation.

Effect of Se(IV) and Se(VI) on the pigment content

The pigment content in plants gives an indication of the physiological changes after Se exposure. The content of pigments (chlorophyll α, chlorophyll β, phaeopigments α, and phaeopigments β) in *Lemna* calculated by equations (2-5) decreased significantly with increasing time of exposure to 5.0 mg/L Se(IV) (Figures 4a and 4b). After 3 d of incubation, the pigment content in *Lemna* exposed to 5.0 mg/L of Se(IV) was 2-3 times less than that of the control, while no significant influence was observed in the first 3 d of growth at 0.5 mg/L Se(IV) exposure (Figure 4a). This indicated that the exposure to high Se(IV) concentration (5.0 mg/L) inhibited the synthesis of

pigments. On the other hand, the Se(VI) amendment did not cause any notable inhibition of the pigment content of *Lemna* compared to the control, although the pigment content of all treatments, including the control, decreased slightly upon prolonging the incubation time (Figures 4c and 4d). These results further confirmed that *Lemna* tolerates Se(VI) better than Se(IV).

In this study, the lower pigment content in *Lemna* upon exposure to 5.0 mg/L of Se(IV) may be due to lipid peroxidation of the chloroplast membranes, resulting in cell damage and photosynthesis disruption. Studies have demonstrated the formation of reactive oxygen species (ROS) in plants under Se exposure, which is reflected in a higher malondialdehyde (MDA) and superoxide radical (O_2^-) content in plants exposed to Se (Dai and Jia, 2017). The produced ROS, including hydroxyl radicals (•OH), superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2), can cause oxidative damage to plant cell structures, cell membranes and photosynthetic pigments (Parlak and Yilmaz, 2012), which could lead to the decreased pigment content. Similarly to the present study, Zhong and Cheng (2016) found that 40-80 µmol/L of Se(IV) (equivalent to 3.2 - 6.4 mg/L Se) decreases the carotenoid and chlorophyll content of the duckweed *Landoltia punctata.* Similarly, the exposure of the duckweed *Lemna minor* to concentrations of Se(IV) exceeding 2 mg/L negatively affected the photochemical efficiency as well as the electron transport system activity (Mechora et al., 2015).



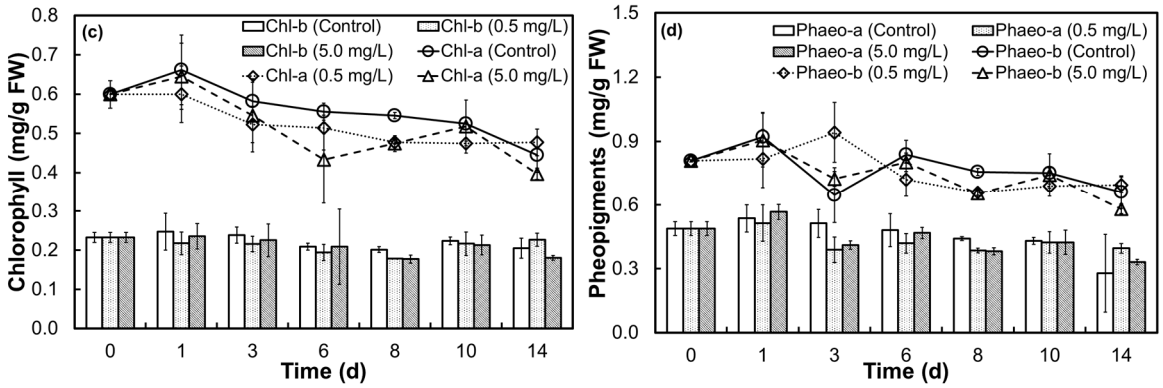


Figure 4. Change in pigments in *Lemna* during 14 days incubation. (a) chlorophyll α and chlorophyll β in the Se(IV) application; (b) phaeopigments α and phaeopigments β in the Se(IV) application; (c) chlorophyll α and chlorophyll β in the Se(VI) application; and (d) phaeopigments α and phaeopigments β in the Se(VI) application.

Effect of Se(IV) and Se(VI) on the Se uptake

The Se content in *Lemna* increased as a function of the Se concentration in the medium, both for the Se(IV) and Se(VI) exposure (Figure 5). *Lemna* accumulated between 4 and 9 times more Se(IV) than Se(VI). For instance, after 1 d of incubation, the Se content in *Lemna* was 431 and 96 mg/kg at 5.0 mg/L Se(IV) and Se(VI) exposure, and 77 and 9 mg/kg at 0.5 mg/L Se(IV) and Se(VI) application, respectively. *Lemna* showed a rapid accumulation of Se during the first 3 d, followed by a slower accumulation phase. Specifically, under 5.0 mg/L Se(IV) and Se(VI) exposure, the Se content of *Lemna* increased, respectively, by 99.7% and 98.8% in the first 3 d of incubation compared with the beginning of the experiment, and by only 5.9% and 7.8% in the remaining 11 d of incubation, compared with the Se content at 3 d.

The higher and faster accumulation of Se(IV) than Se(VI) has also been observed in other plants. For instance, soybean (*Glycine max*) took up 4 times more Se(IV) than Se(VI) after 50 h of exposure to 5.0 μmol/L Se (equivalent to 0.4 mg/L Se) (Zhang et al., 2003) and tomato (*Solanum lycopersicum* L.) accumulated 10-fold more Se(IV) than Se(VI) in roots and shoots when exposed to concentrations of Se higher than 0.5 mg/L (Wang et al., 2019). The higher uptake of Se(IV) than Se(VI) can be explained by their different uptake mechanism and metabolism. Se(IV) is mostly taken up in a faster passive diffusive way and quickly converted

306 into organic Se forms by plants (e.g., SeMet, $SeCys_2$, Me-SeCys and Se-methyl-selenocysteine
307 (SeMetSeCys)) (Arvy, 1993; de Oliveira et al., 2017; Li et al., 2020). In contrast, Se(VI) is
308 taken up in an active way through the facilitation of a S transporter and easily redistributed from
309 the roots to shoots (Arvy, 1993; Li et al., 2008). Afterwards, Se(VI) is reduced to Se(IV) and
310 then converted into organic Se compounds. Se(VI) reduction occurs via substitution of sulfate
311 in the ATP sulfurylase reductase system, which is an ATP-consuming process and the rate-
312 limiting step in the Se(VI) transformation (Van Hoewyk, 2013). The Se(VI) reduction rate is
313 thus much slower than its uptake rate, resulting in Se(VI) saturation and lower accumulation in
314 the plant tissues (Li et al., 2020).

315 Additionally, the plant exposure to high Se concentrations resulted in a transmembrane potential
316 gradient between the inside and outside of the cells. Thus, Se is transported across the cell
317 membrane through ion channels and rapidly enters into the plant cells at the beginning of the
318 exposure (Reid and Hayes, 2003). This could partially explain the fast Se accumulation during
319 the first 3 d of incubation. Likewise, studies have demonstrated that Se(VI) is taken up by plants
320 via sulfate transporters through expression of SULTR1;2, whereas Se(IV) was transported by
321 phosphate transporters present in the root plasma membranes (Gupta and Gupta, 2017; Li et al.,
322 2008). In this study, the fast Se accumulation during the first 3 d of incubation could be partially
323 attributed to a higher amount of transporters in the root plasma membrane at the beginning of
324 the exposure phase, which could support the rapid transportation of Se from the surrounding
325 medium into the plants. Accordingly, the depletion of specific transporters along with the

incubation time could result in a slower accumulation after 3 d. Besides, Se absorption by the plant could also partially contribute to the fast Se accumulation during the first 3 d of growth.

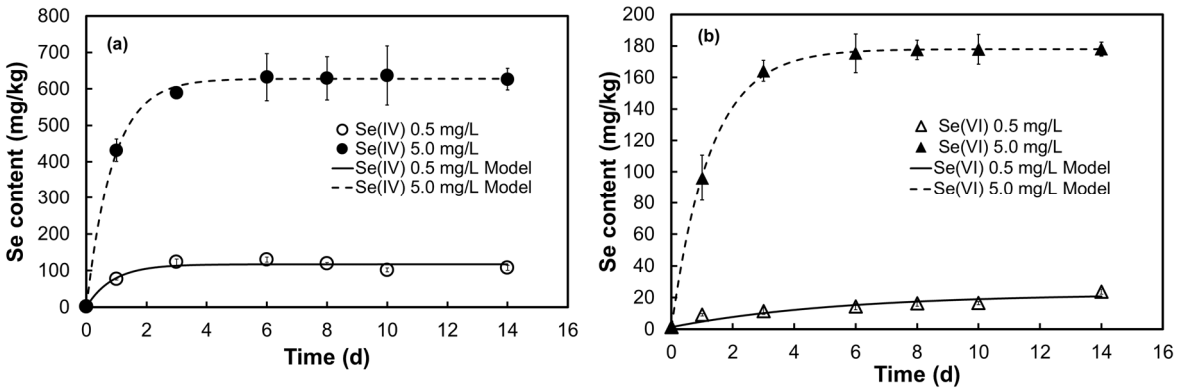


Figure 5. Selenium content of *Lemna minuta* after exposure to different concentrations of (a) selenite and (b) selenate. The symbols represent measured experimental values and the lines represent the two-compartment model fitting

Kinetic model of Se uptake by *Lemna minuta*

The time course of Se uptake and clearance in toxicokinetic studies were analyzed using the two-compartment model (equation (6)) (Clason et al. 2004b). Selenium exposure was considered as the first compartment and *Lemna* as the second compartment. Likewise, a hyperbolic model (equation (8)) was applied to analyze the Se uptake with time by *Lemna*. The estimated parameters of the two-compartment and hyperbolic models are shown in Table 1. Both models provided a good fit to the experimental Se uptake data at low and high Se concentrations. The coefficients of determination (R^2) for the fitting ranged from 0.83 to 0.97 ($P < 0.0001$) in both models, indicating that the two-compartment model and hyperbolic model can be used to estimate the Se uptake by *Lemna.*

Table 1. Kinetic parameters of bioaccumulation of Se in *Lemna* at different Se concentrations according to the fitting to the two-compartment and hyperbolic models.

	Se dosage (mg/L)	Model						
		Two compartment				Hyperbolic		
		R^2	K_a	K_b	BCF (K_a/K_b)	R^2	C_{max}	$t_{max/2}$
Se(IV)	0.5	0.93	263	1.14	231	0.90	123	0.43
	5.0	0.97	142	1.13	125	0.96	670	0.52
Se(VI)	0.5	0.83	8.0	0.18	42	0.85	26	4.63
	5.0	0.99	28	0.79	35	0.97	196	0.89

According to the two-compartment model, both the uptake rate (K_a) and the bioconcentration factor (*BCF*) (obtained by equations (6) and (7)) of Se(IV) in *Lemna* were much higher compared to those of Se(VI) (Table 1). Additionally, the increase in Se concentration affected the uptake rate differently depending on the Se species. The uptake rate decreased from 263 to 142 mg/kg/d upon increasing the Se(IV) dosage from 0.5 to 5.0 mg/L, while it increased from 8 to 28 mg/kg/d with increasing Se(VI) concentration. On the contrary, the *BCF* decreased with increasing Se dosing both in Se(IV) and Se(VI) treatments. The *BCFs* at the theoretical equilibrium were 125 and 231 for Se(IV), and 35 and 42 for Se(VI) at 5.0 and 0.5 mg/L Se dosage, respectively. Ohlbaum et al. (2018) also found that increasing the ambient Se concentration from 0.1 mg/L to 0.5 mg/L decreased the *BCF* values in both *Lemna minor* and *Egeria densa*.

The higher values of the *BCF* and K_a in the Se(IV) treatment confirmed the ability of *Lemna* to accumulate Se(IV) in larger amounts and faster than Se(VI). This partially supports a faster passive uptake of Se(IV), but a slower active uptake of Se(VI) by plants. The *BCF* is generally used to measure the capability of aquatic organisms to bioconcentrate pollutants. The kinetic Se *BCFs* in *Lemna* obtained in the present study are higher than in other plants (Table 1). For example, Dai and Jai (2017) studied the effect of Se on the growth, tolerance and antioxidative system of three alfalfa cultivars and evidenced that the maximum *BCF* of alfalfa is 15.4 at 900 µmol/L Se(VI) (equivalent to 63 mg/L Se). It should be noted that there are two different approaches to calculate the *BCF*: (1) using the ratio of K_a and K_b from kinetic data not assuming

363 that an equilibrium has been reached during the experiment; or (2) using the ratio of the element
364 concentration in the plants and the exposure concentration assuming that an equilibrium has
365 been reached (Clason et al., 2003). The second method is mostly applied to quantify the *BCF*
366 value, resulting in an inaccurate estimation of the accumulation ability of organisms, as the
367 equilibrium state is difficult to reach or takes a long time (Dai and Jia, 2017; Ohlbaum et al.,
368 2018). Although some studies have applied the toxicokinetic model to evaluate the kinetic
369 accumulation and *BCF* of heavy metals, such as Cd, Pb, Cu and Zn, there is no investigation on
370 Se kinetic accumulation and *BCF* evaluation by *Lemna* yet.

371 The maximum Se content in *Lemna* (C_{max}) at the theoretic equilibrium and the time to reach
372 half of the C_{max} ($t_{max/2}$) were obtained from the hyperbolic model. Specifically, the C_{max} was 123
373 and 670 mg/kg for the 0.5 and 5.0 mg/L Se(IV) application, and 26 and 196 mg/kg for the Se(VI)
374 exposure, respectively (Table 1).

375 The suitability of bioaccumulation models is influenced by the involvement of different
376 intracellular element-handling mechanisms in organisms (Clason et al., 2004b). In this study,
377 the Se accumulation with the increasing exposure time followed the saturation curve for both
378 Se species (Figure 5, Table 1), instead of the linear first-order kinetic model. This indicated that
379 both the Se(IV) and Se(VI) uptake by *Lemna* are not only through passive diffusion, but also
380 through other processes such as ion channel proteins or transporters (Clason et al., 2004b). This
381 is in agreement with other studies, which showed that the Se(VI) uptake mechanism is an active
382 process, facilitated by energy and S transporters (Arvy 1993; de Oliveira et al., 2017; Wang et
383 al., 2019), which has been verified by studying the effects of respiratory inhibitors,
384 hydroxylamine and sulfur on the Se uptake by plants (Arvy, 1993; Li et al., 2008). However,
385 the mechanism of Se(IV) uptake by plants remains unclear. Some researchers reported that the
386 Se(IV) uptake is mainly controlled by passive diffusion (Arvy, 1993; de Oliveira et al., 2017).
387 Wang and Dei (1999) studied the kinetics of metal accumulation (Cd, Cr, Se and Zn) in two

388 macroalgae species and verified that metal uptake follows a linear pattern only over a 2 d
389 exposure period, indicating that metal uptake proceeded by passive diffusion. Li et al. (2008)
390 studied the Se(IV) uptake in phosphorus-starved plants and concluded that Se(IV) uptake is an
391 active process likely mediated, at least partly, by phosphate transporters. This partially supports
392 the findings in the present study. Yet, many studies have been investigating the mechanism of
393 Se uptake by organisms through biochemical or genetic methods (El Mehdawi et al., 2018),
394 while only few studies validated the Se uptake mechanism by applying toxicokinetic models.

395 Additionally, together with the experimental data in Figure 5 and the $t_{max/2}$ from the hyperbolic
396 model (Table 1), the Se concentration in *Lemna* apparently reached a relatively steady state
397 after 6 d of exposure to both Se species, which could give a reference to other studies to choose
398 the equilibrium time before implementing a hydroponic experiment in Se-spiked medium or
399 provide the information for some other similar systems to design the size, flow rate and retention
400 time.

401 **Conclusions**

402 This study demonstrated that *Lemna* has a higher tolerance to Se(VI) compared to Se(IV), as
403 shown by the lower root tolerance index of *Lemna* upon Se(IV) exposure and the decrease of
404 pigment content upon 5.0 mg/L Se(IV) exposure. The content of Se in *Lemna* increased with
405 increasing Se exposure concentration for both types of Se oxyanions. The Se content in *Lemna*
406 reached a steady state after 6 d of exposure. The accumulation of Se by *Lemna* could be modeled
407 by both the two-compartment model and the hyperbolic kinetic model, indicating that the uptake
408 of both Se species by *Lemna* is controlled by complex processes. The higher *BCFs* at theoretical
409 equilibrium and faster uptake rates (K_a) obtained with the two-compartment model for the Se(IV)
410 treatment evidenced that *Lemna* rapidly takes up and accumulates Se(IV). These results indicate
411 that *Lemna* could potentially be used as a phytoextraction plant to remove Se(IV) from

412 wastewater, which may eventually also lead to the production of Se-enriched crop fertilizers or
413 protein-rich food/feed supplements.

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